Applicant: Karin A. Eidne et al.

Serial No.: 10/580,130

Attorney's Docket No.: 210040002US1 / SJB:JBD:FP23821

Filed : May 4, 2007
Page : 3 of 13

Amendments to the Claims

This listing of claims replaces all prior versions and listings of claims in the application:

1-2. (Canceled)

- 3. (Currently Amended) A multi-component detection system comprising:
- i). a first agent comprising a first interacting group coupled directly or indirectly to a first tag, which first tag emits a first energy light of a first wavelength upon activation by a substrate or energy source thereby producing which produces a first activated tag;
- ii). a second agent comprising a second interacting group coupled directly or indirectly to a second tag, which second tag can accept the said first energy from the first activated tag in i) when the first and second interacting groups are associated and an appropriate substrate or energy source for the first tag in i) is present thereby producing a second activated tag that emits a second energy light of a second wavelength;
- iii). a third agent comprising a third interacting group coupled directly or indirectly to a third tag that can accept the said first energy from the first activated tag in i) when the first and third interacting groups are associated and an appropriate substrate or energy source for the first tag in i) is present and that can accept the said second energy from the second activated tag in ii) when the second and third interacting groups are associated and an appropriate substrate or energy source for the second tag in ii) is present to produce a third activated tag that emits a third energy light of a third wavelength;
- iv). an appropriate substrate or energy source to activate the tags in i) and ii);
- v).—a means of detecting said <u>first</u>, <u>second or third energy</u>, <u>or combinations</u> thereof, wherein external stimuli are applied to directly or indirectly modulate the association of <u>interacting groups</u> emitted light.

4-6. (Canceled)

Applicant: Karin A. Eidne et al.

Serial No.: 10/580,130

Attorney's Docket No.: 210040002US1 / SJB:JBD:FP23821

Serial No.: 10/580,130 Filed: May 4, 2007 Page: 4 of 13

7. **(Previously Presented)** The system of claim 3, wherein the interacting group is selected from the group consisting a compounds, proteins, protein domains, protein loops, protein termini, peptides, hormones, protein-lipid complexes, lipids, carbohydrates, carbohydrate-containing compounds, nucleic acids, oligonucleotides, pharmaceutical agents, pharmaceutical drug targets, antibodies, antigenic substances, viruses, bacteria, and cells.

- 8. **(Previously Presented)** The system of claim 3, wherein the interacting group is selected from the group consisting of carbohydrates, proteins, drugs, chromophores, antigens, chelating compounds, molecular recognition complexes and combinations thereof.
- 9. **(Previously Presented)** The system of claim 7, wherein the nucleic acid molecule comprises DNA including single-stranded and double-stranded DNA, RNA including heterogeneous ribonucleic acid (hnRNA), transfer ribonucleic acid (tRNA), messenger ribonucleic acid (mRNA), or ribosomal ribonucleic acid (rRNA).

10. (Canceled)

- 11. **(Currently Amended)** The system of claim <u>3</u> [[10]], wherein the external stimuli are reagents comprising organic and inorganic molecules, proteins, nucleic acids, carbohydrates, lipids, ligands, drug compounds, agonists, antagonists, inverse agonists or compounds.
- 12. **(Currently Amended)** The system of claim $\underline{3}$ [[10]], wherein the external stimuli are changes of conditions including temperature, ionic strength or pH.
- 13. **(Previously Presented)** The system of claim 3, wherein the detection tag is selected from the group consisting of a bioluminescent protein, a fluorescent protein, a fluorescent moiety or a non-fluorescent quencher.

Applicant : Karin A. Eidne et al.

Serial No. : 10/580,130

Attorney's Docket No.: 210040002US1 / SJB:JBD:FP23821

Filed : May 4, 2007 Page : 5 of 13

14. **(Previously Presented)** The system of claim 13, wherein the bioluminescent protein is selected from the group consisting of luciferase, galactosidase, lactamase, peroxidase or any protein capable of luminescence in the presence of a suitable substrate.

- 15. **(Previously Presented)** The system of claim 13, wherein the fluorescent protein is selected from the group consisting of green fluorescent protein (GFP) or variants thereof, blue fluorescent variant of GFP (BFP), cyan fluorescent variant of GFP (CFP), yellow fluorescent variant of GFP (YFP), enhanced GFP (EGFP), enhanced CFP (ECFP), enhanced YFP (EYFP), GFPS65T, Emerald, Topaz, GFPuv, destabilised EGFP (dEGFP), destabilised ECFP (dECFP), destabilised EYFP (dEYFP), HcRed, t-HcRed, DsRed, DsRed2, t-dimer2, t-dimer2(12), mRFP1, pocilloporin, Renilla GFP, Monster GFP, paGFP, Kaede protein and kindling protein, Phycobiliproteins and Phycobiliprotein conjugates including B-Phycocrythrin, R-Phycocrythrin and Allophycocyanin or any other protein capable of fluorescence.
- 16. **(Previously Presented)** The system of claim 13, wherein the fluorescent moiety is selected from the group consisting of Alexa Fluor dyes and derivatives, Bodipy dyes and derivatives, Cy dyes and derivatives, fluorescein and derivatives, dansyl, umbelliferone, fluorescent and luminescent microsheres, fluorescent nanocrystals, Marina Blue, Cascade Blue, Cascade Yellow, Pacific Blue, Oregon Green and derivatives, Tetramethylrhodamine and derivatives, Rhodamine and derivatives, Texas Red and derivatives, rare earth element chelates or any combination or derivative thereof or any other molecule with fluorescent properties.
- 17. **(Previously Presented)** The system of claim 13, wherein the non-fluorescent quencher is selected from the group consisting of dabcyl, non-fluorescent pocilloporins, QSY-7, QSY-9, QSY-21, QSY-35, BHQ-1, BHQ-2, BHQ-3 or any known non-fluorescent chromophore with the ability to absorb light and to quench fluorescence and/or luminescence.
- 18. **(Currently Amended)** The system of claim 3, wherein the <u>energy source to</u> <u>activate the tags in i) and ii) activation energy</u> is generated by a light source including lasers, Hglamps, Xe-lamps and halogen lamps or any light emitting device, and the generated light is

Applicant : Karin A. Eidne et al.

Serial No. : 10/580,130

Attorney's Docket No.: 210040002US1 / SJB:JBD:FP23821

Filed : May 4, 2007
Page : 6 of 13

restricted to a specific wavelength or wavelength range by appropriate means including laser lines, bandpass filters, shortpass filters, monochromators or any device to spectrally restrict the emitted light.

- 19. **(Currently Amended)** The system of claim 3, wherein the <u>appropriate substrate to activate the tags in i) and ii)</u> first activation energy is generated from a suitable enzyme substrate selected from the group consisting of coelenterazine and luciferin or a derivative derived therefrom.
- 20. (Currently Amended) The system of claim 3, wherein sequentially the first detection tag is activated and the <u>energy light</u> emitted from the first, second and third <u>activated</u> detection tag is detected and then the second detection tag is activated and the <u>energy light</u> emitted from the second and third detection tag is detected.

21. (Canceled)

- 22. **(Previously Presented)** The system of claim 20, wherein the sequence of activation and detection is repeated over time to yield temporal information.
- 23. **(Previously Presented)** The system of claim 3, wherein the interacting group and tag are coded for in a fusion protein construct.
- 24. **(Previously Presented)** A recombinant DNA encoding the fusion protein construct of claim 23.
- 25. **(Previously Presented)** A fusion gene that comprises the recombinant DNA of claim 24.
- 26. **(Previously Presented)** A DNA cassette comprising a promoter operably linked to one or more fusion protein genes of claim 23.

Applicant: Karin A. Eidne et al. Attorney's Docket No.: 21004-0002US1 / SJB:JBD:FP23821

Serial No.: 10/580,130 Filed : May 4, 2007 : 7 of 13

Page

27. (Previously Presented) A vector comprising the fusion gene of claim 25.

28. A host cell transiently or stably transformed or transfected (Previously Presented) by a vector of claim 27.

- 29. (Previously Presented) The host cell of claim 28, wherein the cell is human, mammalian, insect, plant, bacterial, or yeast.
- 30. (Previously Presented) The vector of claim 27, wherein the gene construct is under the control of a constitutive promoter.
- 31. (Previously Presented) The vector of claim 27, wherein the gene construct is under the control of an inducible promoter.
- 32. (Previously Presented) The vector of claim 30, wherein the constitutive promoter is selected from the group consisting of CMV, SV40, RSV, EF-1 a, Tk, and AdML, when the cell to be transformed or transfected is mammalian.
- 33. (Previously Presented) The vector of claim 30, wherein the constitutive promoter is selected from the group consisting of T7, AraBAD, trc, pL, tac, and lac, when the cell to be transformed or transfected is a bacterial cell.
- 34. (Previously Presented) The vector of claim 30, wherein the constitutive promoter is selected from the group consisting of nmtl, gall, gallo, TEF1, AOX1, GAP, and ADH1, when the cell to be transformed or transfected is a yeast cell.
- 35. (Previously Presented) A virus comprising the fusion gene of claim 25.
- 36. (Previously Presented) A host cell infected by the virus of claim 35.

Applicant: Karin A. Eidne et al.
Serial No.: 10/580,130

Attorney's Docket No.: 21004-0002US1 / SJB:JBD:FP23821

Serial No.: 10/580,130 Filed: May 4, 2007 Page: 8 of 13

37-45. (Canceled)

46. **(New)** The system of claim 3, wherein the energy emitted by the first, second and third activated tags comprises resonance energy, light energy, or both, specific for each of the tags.